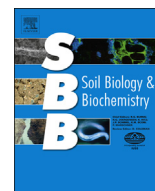


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Microbial community structure mediates response of soil C decomposition to litter addition and warming

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ABSTRACT

Microbial activity has been highlighted as one of the main unknowns controlling the fate and turnover of soil organic matter (SOM) in response to climate change. How microbial community structure and function may (or may not) interact with increasing temperature to impact the fate and turnover of SOM, in particular when combined with changes in litter chemistry, is not well understood. The primary aim of this study was to determine if litter chemistry impacted the decomposition of soil and litter-derived carbon (C), and its interaction with temperature, and whether this response was controlled by microbial community structure and function. Fresh or pre-incubated eucalyptus leaf litter (¹³C enriched) was added to a woodland soil and incubated at 12, 22, or 32 °C. We tracked the movement of litter and soil-derived C into CO₂, water-extractable organic carbon (WEOC), and microbial phospholipids (PLFA). The litter additions produced significant changes in every parameter measured, while temperature, interacting with litter chemistry, predominately affected soil C respiration (priming and temperature sensitivity), microbial community structure, and the metabolic quotient (a proxy for microbial carbon use efficiency [CUE]). The direction of priming varied with the litter additions (negative with fresh litter, positive with pre-incubated litter) and was related to differences in the composition of microbial communities degrading soil-C, particularly gram-positive and gram-negative bacteria, resulting from litter addition. Soil-C decomposition in both litter treatments was more temperature sensitive (higher Q₁₀) than in the soil-only control, and soil-C priming became increasingly positive with temperature. However, microbes utilizing soil-C in the litter treatments had higher CUE, suggesting the longer-term stability of soil-C may be increased at higher temperature with litter addition. Our results show that in the same soil, the growth of distinct microbial communities can alter the turnover and fate of SOM and, in the context of global change, its response to temperature.

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1. Introduction

Human activity has transformed our soils and landscapes, and global change phenomena such as warming temperatures, increasing CO₂ concentrations, and N deposition will continue as population pressures increase and climate change continues (Vitousek et al., 1997). Maintaining or increasing global soil C stocks is desirable for soil function and agricultural productivity, but also as a climate change mitigation strategy (Lal, 2004). Despite intensive research on these topics, there is no consensus as to how climate change, largely through the effects of warming

temperatures, nitrogen availability, and plant productivity, will impact soil and litter decomposition and ecosystem carbon stocks (Curtis and Wang, 1998; Neff et al., 2002; Mack et al., 2004; Pregitzer et al., 2008; Fernández-Martínez et al., 2014).

The importance of physical protection to the stabilization of soil organic matter (SOM) has been understood for decades (Sollins et al., 1996; Krull et al., 2003; Dungait et al., 2012), as has the importance of plant litter chemistry upon the extent of its decay (Melillo et al., 1982; Taylor et al., 1989). However, the role microbial community structure and function plays in soil and litter turnover, and its response to elevated temperatures and N availability, is less understood. The concept of a functional redundancy of microbial communities, where under the right environmental circumstances the same functions and processes can be performed regardless of the microbial community present in the soil, has recently been

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challenged (Allison and Martiny, 2008; Strickland et al., 2009), and changes to microbial community structure have been linked to a wide range of ecosystem processes (Wardle et al., 2004; Balser and Firestone, 2005; Lau and Lennon, 2012; De Vries et al., 2013). For example, distinct microbial communities can impact the resulting chemistry and mass loss of litter (Wickings et al., 2012; Allison et al., 2013), particularly if the litter is of similar composition to that which is frequently experienced by the microbes (Ayres et al., 2009; Freschet et al., 2012). However, whether changes in litter decomposition will ultimately impact downstream soil-C dynamics is uncertain (Kemmitt et al., 2008; Schimel and Schaeffer, 2012; Cleveland et al., 2014). Priming, the increased loss of soil-C due to the addition of labile substrates, has also been related to changes in microbial community structure (Fontaine et al., 2003; De Graaff et al., 2010; Blagodatskaya et al., 2014), and provides a link between the quantity or quality of more thermodynamically labile inputs and the potential destabilization of soil carbon (Guenet et al., 2012; Wild et al., 2014).

When trying to understand the interactions between microbial community structure, C decomposition, C quality, and the global change drivers of temperature and N availability, the picture becomes even less clear. Despite extensive work on the subject, the degree to which C chemistry alone drives the decomposition of litter and soil in response to elevated temperatures is frequently debated (Giardina and Ryan, 2000; Bol et al., 2003; Fierer et al., 2005; Knorr et al., 2005; Bauer et al., 2008; Conant et al., 2008; Craine et al., 2010; Hopkins et al., 2012; Suseela et al., 2013; Lefevre et al., 2014), as is the response of soil-C to N addition (Melillo et al., 2002; Neff et al., 2002; Auyeung et al., 2013). Part of this confusion may lie in the impact and influence of microbial community structure and function, as it has been shown as a potential driver of soil C turnover in response to N (Billings and Ziegler, 2008; Chen et al., 2014; Kaiser et al., 2014), warming (Bardgett et al., 2008; Balser and Wixon, 2009; Yuste et al., 2011; Zhou et al., 2012; Nie et al., 2013; Ziegler et al., 2013; Hopkins et al., 2014; Wei et al., 2014), and the interactive effects of N and warming (Cusack et al., 2010; Li et al., 2013).

Research into the impact of microbial communities upon soil and litter dynamics underscores the importance in fully understanding this topic to predict changes in C stocks to environmental perturbations. SOM models that include microbial community structure have shown that changes in microbial physiology, often expressed as CUE (defined as the amount of C in microbial biomass relative to C respired and in biomass), have the potential to dramatically impact the fate of C stocks to climate change (Allison et al., 2010; Schimel, 2013; Wieder et al., 2013; Li et al., 2014), and experimental evidence shows that CUE can be altered by substrate quality, temperature, and N availability (Dijkstra et al., 2011; Manzoni et al., 2012; Frey et al., 2013; Tucker et al., 2013). This concept of changes to CUE is of particular importance as a large portion of SOM has been attributed to microbial derived products, which may have a relatively long turnover time (Simpson et al., 2007; Liang and Balser, 2008; Miltner et al., 2012; Gleixner, 2013). It is therefore necessary to determine the underlying mechanisms and the extent that microbial community structure and function impacts C turnover in response to these global change scenarios.

The overarching purpose of this study was to determine whether the addition of litters of differing thermodynamic stability to the same soil impacted the response of soil-C to short-term warming, and whether this response was controlled by microbial community structure and function. The novelty of this study lies in the use of a strong (^{13}C) isotopic label to partition all measured C pools (WEOC, PLFA, CO_2) accurately into soil- and litter-derived pools, and therefore attribute soil and litter decomposition (and

its response to temperature) to particular microbial groups. By using the same soil in the incubations, any changes in the response of soil-C decomposition to the litter addition could be more directly linked to the microbial community. We first hypothesized that despite using the same soil in all treatments, the microbial communities degrading soil-C would differ between the litter treatments and the no-litter control due to established impacts of C chemistry and availability upon microbial community structure (e.g. Allison et al., 2013). We hypothesized that the addition of both litters would result in a greater release of soil-C relative to the no litter control (positive priming), but that the more thermodynamically stable litter would produce a stronger priming effect due to its greater chemical similarity to the soil (e.g. Fontaine et al., 2003). Finally, we hypothesized that differing microbial communities degrading the same source of soil-derived C would alter the response of soil-C mineralization (and assimilation) to temperature due to observed impacts of microbial community structure upon the response to warming (e.g. Zhou et al., 2012).

2. Materials and methods

2.1. Soil and litter

Soils were sampled in Sept 2012 from a remnant native woodland ecosystem within the Glen Rock farm in Yass, New South Wales, Australia. For further information regarding soil characterization and site descriptions see De Menezes et al. (2014). After removing surface residues, two 10-cm deep soil cores were taken from 10 randomly selected sites along a 10 × 10 m grid to capture the heterogeneity of the site. Soils were placed on ice immediately after sampling. All soil cores were combined, homogenized, and sieved to 5 mm.

Fresh eucalyptus (*Eucalyptus globulus*) leaf litter with a ^{13}C fraction, $\delta^{13}\text{C}$, of 21.5‰ was produced through continuous labeling with ^{13}C enriched CO_2 over four months in a labeling cabinet (Butler and Eickhoff, 1979). Ammonium nitrate was added as an N source during growing. This litter source is referred to as “fresh litter” in this manuscript. A subsample of the fresh leaf litter was incubated for 11-weeks in the dark at 25 °C and extracted each week with 0.01 M CaCl_2 ; the ^{13}C fraction of the remaining material was 21.4‰ (Table 1). The CaCl_2 extracted, 11-week incubated leaf litter is referred to as “pre-incubated litter” in this manuscript. See Sanderman et al. (2014) for details regarding the production of the pre-incubated litter.

2.2. Experimental design

In this experiment the fresh litter was used as a higher quality litter, and the pre-incubated litter as a lower quality litter. The difference in ‘qualities’ of the litters predominately refers to the kinetic stability of the compounds in the litters, as the fresh litter was comprised of more labile carbohydrates, and the pre-incubated litter of more stable lipids (Table 1), consistent with changes in litter upon decomposition (Baldock et al., 1997). While higher C:N or lignin:N ratios of fresh litter are often related to slower decomposition and lower stability (Melillo et al., 1982; Taylor et al., 1989), in this instance the lower C/N (and lignin/N) ratio of the pre-incubated litter corresponds to a higher loss of C relative to N during decomposition, and therefore does not indicate a substrate with the potential for greater decomposition. While focusing on just one type of litter, this design had advantages over using a variety of litters with contrasting ‘quality’ as the litter only pre-incubation (described in Sanderman et al., 2014), ensures that the remaining chemistry of the pre-incubated litter has greater kinetic stability. Finally, eucalyptus woodland soils and litters are not often

Table 1Characteristics of the two *E. globulus* litters and the eucalyptus woodland soil used in the incubations.

C source	%C	%N	C:N	Modeled molecular composition (% of total C)				
				Carbohydrate	Protein	Lignin	Lipid	Carbonyl
Fresh litter	37.1	2.06	18.0	39.5%	5.0%	34.4%	5.3%	15.8%
Pre-incubated litter	43.3	5.40	8.0	15.3%	6.6%	37.3%	23.8%	18.8%
Soil	2.05	0.073	28.1	26.3%	1.6%	37.6%	18.8%	15.6%

used in warming and N deposition experiments (Von Lützw and Kögel-Knabner, 2009) despite their prevalence across Australia and potential sensitivity to climate change (Prior and Bowman, 2014), and therefore represent an important research gap.

Thirteen grams of fresh, field-moist woodland soil was weighed into 50 mL centrifuge tubes and wetted to 60% water holding capacity (WHC) with sterilized, 18 Ohm H₂O and sealed with Subaseal septa (Sigma Aldrich). The woodland soils were pre-incubated in the dark at 22 °C for 14 days to minimize disturbance from processing. After pre-incubation, one of the two eucalyptus litters were mixed into the soil (10 mg litter-C per g soil-C), and the soil + litter mixtures were incubated for an additional 14 days at 22 °C to deplete the most labile forms of C. No-litter control soils were also mixed and incubated in the same fashion as the litter additions. The incubation temperature was then kept at 22 °C, increased to 32 °C, or decreased to 12 °C for an additional 14 days. The quantity and isotopic composition of respired CO₂ was measured after litter addition (day 0) on days 1, 3, 5, 7, 10, 14, 15, 17, 19, 21, 24, 26, and 28. The microcosm headspace was flushed ten times with humidified, CO₂-free air, and CO₂ was allowed to accumulate until the subsequent sampling time point (after sampling the headspace flushing and CO₂ accumulation was repeated). Complete evacuation of headspace CO₂ was confirmed with four randomly selected samples. After CO₂ accumulation, between 1 and 10 mL of headspace was removed with a gastight syringe and injected into helium-flushed 12 mL exetainer vials (Labco, Ceredigion, UK) and analyzed as described below. Samples were given 1 h to equilibrate to the new incubation temperature (on day 14) before flushing and CO₂ accumulation. Soils were maintained at 60% WHC capacity during the incubation by keeping the tubes sealed and flushing with humidified CO₂-free air; this was checked by periodic weighing of the samples during the incubation and measuring the water content of the soils at destructive sampling time points (see below).

Destructive samples (with four replicates per temperature × litter treatment) were taken on days 0, 14, 15, 19, and 28. Microbial PLFAs (quantity and ¹³C fraction), WEOC (quantity and ¹³C fraction), water-extractable organic nitrogen (WEON), dissolved inorganic N (DIN), and water content were measured on each of the destructive samples (described below). In addition, terminal restriction fragment length polymorphism (T-RFLP) profiles of bacterial communities (16S) were generated and actinobacterial cellulase (glycoside hydrolase family 48) gene numbers quantified on the destructive samples. The cellulase gene in particular was quantified due to the high quantities of cellulose in plant litter and its importance in plant litter decomposition (Kögel-Knabner, 2002). Soil only controls were also measured for CO₂ and destructively sampled along with the litter treatments.

2.3. Analytical procedures

2.3.1. ¹³C NMR

The initial soil and litter were characterized with solid-state ¹³C cross polarization magic angle spinning (CP/MAS) NMR spectroscopy using a Bruker 200 Avance spectrometer equipped with a 4.7 T wide-bore superconducting magnet with a resonance frequency of 50.33 MHz. Spectra were obtained using a 3.2 ms 195 w 90° pulse

with a contact time of 1 ms and a recycle delay of 1 s, set after determining the T1H value on these samples. Details of operating conditions can be found in Baldock et al. (2013). Between 1000 (litters) and 60,000 (soil) scans were used and line broadening of 50 Hz was applied to the soil spectra. Chemical shift regions were defined relative to the methyl resonance of hexamethylbenzene at 17.36 ppm as follows: alkyl-C (0–45 ppm), N-alkyl/methoxyl-C (45–60 ppm), O-alkyl-C (60–95 ppm), di-O-alkyl C (95–110 ppm), aryl-C (110–145 ppm), O-aryl C (145–165 ppm), amide/carboxyl C (165–190 ppm) and ketone-C (190–215). Integration areas within these chemical shift regions were inputted into a five component molecular mixing model to determine proportional quantities of carbohydrate, protein, lignin, lipid, and carbonyl C (Baldock et al., 2004).

2.3.2. CN and isotopes of starting material

The isotopic composition and CN content of the natural abundance (soil) and ¹³C enriched (litter) samples were measured on an ANCA solid/liquid elemental analyzer (EA) interfaced to a GEO 20/20 isotope ratio mass spectrometer (IRMS) (Sercon; Cheshire UK) in continuous flow mode. All natural abundance measurements are reported using standard δ notation relative to VPDB (Coplen, 1994). Enriched (¹³C) samples were measured using head amplifiers with lower resistance on mass 45 and are reported using atom fraction notation (Coplen, 2011). Percent C and N of the soil and litter were confirmed via high temperature oxidation on a Trueman CN analyzer (Leco Corp; Castle Hill, NSW Australia). All samples were air dried and ground to a fine powder prior to analysis. Effervescence tests with 4 M HCl confirmed the absence of carbonates in the soils.

2.3.3. Respired CO₂

The quantity and isotopic composition of both natural abundance (soil only controls) and ¹³C enriched (soil + litter) CO₂ was measured using a Cryoprep trace gas analyzer (Sercon; Cheshire UK) interfaced to the GEO 20–20 IRMS. Control sample CO₂ concentrations were quantified relative to a six-point calibration curve of CO₂ standards of known concentrations, and the $\delta^{13}\text{C}$ values corrected (value and drift) using carbonate standards (NBS 19 = +1.95‰, IAEA-CO-9 = −47.32‰) digested with an excess of 12 M HCl and a CO₂ tank standard (−11.51‰). Analytical precision was ±0.16‰ (drift corrected).

The ¹³C atom fraction of CO₂ from the soil + litter mixtures was corrected (value and drift) using eight CO₂ standards varying from 2 to 22 ‰ $\delta^{13}\text{C}$, and CO₂ quantified using a six-point calibration curves of standards nearest to those of the samples. The standards were created by mixing 99% $\delta^{13}\text{C}$ Na₂CO₃ with natural abundance Na₂CO₃ ($\delta^{13}\text{C}$ = +1.42‰), digesting with an excess of 12 M HCl, and removing aliquots of the headspace. Analytical precision was ±0.35%.

2.3.4. Water-extractable OM

Dissolved organic matter (DOM) was extracted from soil and soil + litter by shaking for 30 min at a 1:5 ratio (w/v) with cold (4 °C) milliQ water (Rousk and Jones, 2010) followed by filtration (Whatman #42) and freezing. WEOC and total dissolved N (TDN) were quantified using a Thermalox TOC/TN analyzer (Analytical

Sciences; Cambridge, UK). Nitrate + nitrite (Miranda et al., 2001) and ammonium (Mulvaney, 1996) were quantified colorimetrically using a microplate reader (SynergyMX Biotek; Winooski, VT USA). Dissolved organic nitrogen (DON) was calculated as the difference between TN and inorganic N (nitrate and ammonium).

The isotopic composition of WEOC from the controls ($\delta^{13}\text{C}$) and litter treatments (^{13}C atom fraction) were measured by interfacing the Thermalox to the GEO 20/20 IRMS through the Cryoprep (De Troyer et al., 2010). $\delta^{13}\text{C}$ values were corrected (value and drift) with six-point calibration curves of glycine (-40.71%) and sucrose (-10.5%) (analytical precision = 0.43%). ^{13}C atom fraction values were corrected (value and drift) with standards ranging from 2 to 20 ‰ created by mixing 99 ‰ $x(^{13}\text{C})$ Na_2CO_3 with natural abundance Na_2CO_3 ($\delta = +1.42\%$) with an analytical precision of $\pm 0.5\%$.

2.3.5. Microbial PLFA

Soils were flash frozen in liquid nitrogen, freeze-dried, then extracted for PLFA (White et al., 1979; Frostegård et al., 1991). Briefly, 4 g of soil were sonicated for 2 h in a 1:2:0.8 mixture (vol) of chloroform: methanol: citrate buffer (0.15 M, pH 4.0). The organic and aqueous phases were separated with additional chloroform and citrate buffer, and phospholipids were eluted from the organic phase with methanol through Supelclean™ silica columns (Supelco). A 1/10th split of the phospholipids were used for quantification with gas chromatography-mass spectrometry (GC–MS) and the remainder for isotopic analysis with GC-combustion-IRMS (GC-c-IRMS). Fatty acid methyl esters (FAMES) were formed by mild alkaline methanolysis and re-suspended in an internal standard (methyl decanoate). FAMES were quantified relative to a 26-component standard (bacterial acid methyl esters [BAME] mix, Supelco, Bellefonte PA USA) on an HP 6890/5973 (Agilent Technologies Inc; Santa Clara, CA USA) equipped with a 60 m Rxi-5MS column (Restek; Bellefonte, PA USA). The GC program was a splitless injection with a 12 mL min^{-1} purge flow after a 1.00 min sampling time holding the oven at 50°C . Column flow then increased to 1.2 mL min^{-1} and oven temperature ramped from 50 to 150°C at $10^\circ\text{C min}^{-1}$, from 150 to 240°C at 5°C min^{-1} , and then held 240°C for 5 min for a total runtime of 46 min.

Individual PLFA are named using standard nomenclature. In some instances we grouped individual PLFA: general microorganisms (14:0, 15:0, 16:0, 17:0, 18:0), gram-positive bacteria (iso- and anteiso-branched PLFA), gram-negative bacteria (unsaturated and cyclopropyl PLFA), and fungi (18:2 ω 6,9c and 18:1 ω 9) (Zelles, 1999; Ruess and Chamberlain, 2010; Frostegård et al., 2011). We included both cyclopropyl bacteria and unsaturated PLFA in the gram-negative group, and iso- and anteiso-branching in the gram-positive group, as these PLFA differentially alter their quantities based upon a physiological response to warming, so inclusion of both sets within each functional group should provide an indication of changes in microbial community structure, rather than in physiology (Wixon and Balser, 2013). Actinomycetes were not included in groupings due to co-elution during isotopic analyses (see below).

The isotopic composition of individual FAMES were determined using a Trace GC with combustion interface at 940°C and a MAT253 IRMS (Thermo Scientific; Bremen, Germany) using the same column and GC parameters for quantitative analysis (compounds were identified by retention time). Of the 29 compounds quantified, all but two were at sufficient concentrations (i14, br16), and all but four (16:1 ω 7c + 16:1 ω 7t; 17:1 ω 7 + 10Me16:0) had sufficient baseline separation for individual isotopic analysis. The two co-eluting gram-negative biomarkers (16:1 ω 7c + 16:1 ω 7t) were included in the gram-negative grouping while the co-eluting gram-negative and actinomycetes biomarkers were omitted from grouping. The $\delta^{13}\text{C}$ values of the controls (natural abundance) were

corrected relative to a 5-pt calibration ($7\text{--}220 \text{ ng } \mu\text{L}^{-1}$) of an isotopically verified n-alkane standard (A4, Indiana University) while the ^{13}C enriched FAMES were corrected with 6 point calibrations of the $x(^{13}\text{C})$ 2–20% CO_2 standards injected through the Trace GC.

2.3.6. DNA

A 2 g subsample was flash frozen in liquid nitrogen and kept at -80°C until analysis. DNA and RNA co-extractions were performed using RNA PowerSoil® total RNA isolation kit (Mobio, Carlsbad, CA), and DNA was purified following the manufacturer's protocol. Bacterial 16S rRNA gene was amplified using primers 27f (Lane, 1991) and 519r (Lane et al., 1985). The forward primer was labeled with 6-Carboxyfluorescein at the 5' end. The cycling conditions used for 16S rRNA gene amplification was as follows: 94°C for 2 min (one cycle), 98°C for 30 s, 55°C for 30 s, 72°C for 30 s (28 cycles), and a final extension step of 72°C for 10 min. After amplification PCR products were cleaned with MultiScreen filter plates (Millipore, Billerica, MA) and quantified using Quant-iT™ Picogreen® dsDNA quantification kit (Life Technologies™, Mulgrave, Australia) according to the manufacturer's instructions and DNA concentration was normalized to $0.2 \text{ ng } \mu\text{L}^{-1}$. For restriction digest, 20 ng of PCR product was added to a reaction mixture containing sterile water, 20 units of restriction enzyme AluI (New England Biolabs, Ipswich, MA) and 12 μL of the specified buffer and reaction mixtures were incubated at 37°C for 3 h. Digests were desalted with 75% isopropanol precipitation (Sigma–Aldrich, Sydney, Australia). Digested and purified PCR products were re-suspended in nuclease-free sterile water and added to a 10 μL reaction mixture containing 9.7 μL of M Hidi formamide and 0.3 μL of GeneScan™ 600 LIZ size standard (Applied Biosystems, Mulgrave, Australia) and denatured at 94°C for 3 min. Fragment lengths were determined by electrophoresis using an AB3031xl Genetic Analyzer (Applied Biosystems). Restriction fragment profiles were obtained from GENEMAPPER® (Applied Biosystems, Mulgrave, Australia). A custom R script (R Development Core Team, 2010) was used to remove spurious baseline peaks (peaks were removed if they were smaller than the minimum height of 20 fluorescence units, and smaller than 2 times the standard deviation calculated over all peaks). Binning of the resulting fragments was performed using the Interactive Binner R script (Ramette, 2009) with a sliding window approach. Parameters used were a minimum and maximum size cut-offs of 40 and 500 bp respectively, minimum RFI (relative fluorescence intensity) of 0.09, a window size of 2 bp and a shift size of 0.2 bp.

2.3.7. Quantitative PCR

Quantitative PCR was performed to quantify bacterial 16S rRNA and the actinobacterial glycoside hydrolase family 48 (GH48) genes. For the 16S rRNA gene the primers used were Eub338 (Lane, 1991) and Eub518 (Muyzer and Waal, 1993). For the actinobacterial GH48 gene, qPCR primers were developed based on sequences obtained from Genbank and also from sequences amplified from soil. Cloning and sequencing of qPCR amplification products was performed to confirm primer specificity. Glycoside hydrolase 48 family has been shown to function as a cellulase in almost all bacteria, and it is most often found as a single copy in bacterial genomes (Sukharnikov et al., 2012), making it an ideal target for the development of molecular tools to quantify bacterial cellulase abundance.

The 16S rRNA and GH48 genes were quantified in triplicate reactions using the CFX96™ Real Time Detection System (Bio-Rad, Hercules, CA). Cycling conditions were as follows: for 16S rRNA gene, 95°C for 1 min (one cycle), 95°C for 15 s and 56°C for 20s (39 cycles); for GH48, 95°C for 1 min (one cycle), 95°C for 5 s and 57°C

for 20 s (39 cycles). A melt curve from 65 °C to 95 °C was added at the end of the amplification cycles for both genes analyzed. Standards were run in triplicates in each qPCR plate with 10-fold dilution series from 10^8 to 100 copies μL^{-1} of purified PCR product from *Pseudomonas fluorescens* strain 5.2 (16S rRNA gene) or from a local soil actinomycete isolate (GH48 gene) (de Menezes et al., 2014). Amplification efficiencies were >90% and R^2 were >0.99 for all bacterial and GH48 calibration curves.

2.4. Calculations and statistics

2.4.1. Isotopic corrections and mixing models

In all C pools measured (CO_2 , WEOC, PLFA) the contributions of litter and soil-derived C to the total measured C were calculated using a mixing model:

$$x(^{13}\text{C})_T \times (m_S + m_L) = m_S \times x(^{13}\text{C})_S + m_L \times x(^{13}\text{C})_L \quad (1)$$

where the subscripts T, S and L correspond to the total-C, soil-C and litter-C (respectively), m corresponds to molar quantities, and $x(^{13}\text{C})$ to atom fractions. As the molar quantities from the soil and the litter by definition equal the molar quantities of total C:

$$m_T = m_S + m_L \quad (2)$$

Equation (2) can be substituted into Equation (1), and then solved for m_L to determine the proportional contribution of litter-C to total C:

$$m_L = \frac{(m_T \times x(^{13}\text{C})_T - m_T \times x(^{13}\text{C})_S)}{(x(^{13}\text{C})_L - x(^{13}\text{C})_S)} \quad (3)$$

In this mixing model, the atom fraction values measured from the control soil were used as the $x(^{13}\text{C})_S$ end members while the atom fraction value of the litter itself was used as the $x(^{13}\text{C})_L$ end member. Therefore this model assumes that any isotopic fractionation between the litter source and CO_2 , WEOC, and PLFA formation, likely on the order of 1–6 ‰ (Fernandez and Cadisch, 2003; Hobbie and Werner, 2004), as well as any potential fractionation from the temperature treatments, have a negligible contribution to the mixing model. Due to the >23,000‰ difference between the litter and soil end members, this assumption is valid.

The proportional quantities of litter-derived C were then multiplied by measured total C yields (as CO_2 , WEOC, or PLFA) to determine the amount of litter-derived C in each C pool. Soil-derived C was determined by subtracting litter-C from total-C. For PLFA, the proportional mol% contribution of an individual PLFA to the soil or litter PLFA pool was determined by dividing the litter (or soil) PLFA yield for an individual PLFA by the total litter (or soil) PLFA within a sample.

Prior to inclusion in the mixing model Equation (3), the $\delta^{13}\text{C}$ and $x(^{13}\text{C})$ values of PLFA were corrected for the contribution of the methyl group added during methanolysis ($\delta^{13}\text{C} = -37.03\text{‰}$, $n = 10$ measured by EA-IRMS) with the following formula:

$$x(^{13}\text{C})_{\text{PLFA}} = \frac{(N \times x(^{13}\text{C})_{\text{PLFA+ME}} - x(^{13}\text{C})_{\text{MeOH}})}{(N - 1)} \quad (4)$$

where the subscript PLFA is the phospholipid fatty acid, MeOH the added methyl group, PLFA + ME the phospholipid methyl ester, and the variable N the total number of C atoms in the phospholipid methyl ester. Equation (4) was derived through rearrangement of the general mixing model Equation (1).

2.4.2. Calculations

A two-pool exponential decay model was fitted to the cumulative percent of litter-C and soil-C remaining at 22 °C during the two week pre-incubation and four week incubation (Collins et al., 2000; Paul et al., 2001):

$$S(t) = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \quad (5)$$

where $S(t)$ is the % of litter-C or soil-C remaining at time t , a_1 and k_1 are the size and decay constant of the more actively cycling portion of C, and a_2 and k_2 are the size and decay constant of the more slowly cycling portions of C. The mean residence time (MRT) of the pools is the inverse of the turnover rate ($1/k$). Curves were fitted to the 2 week incubation at 22 °C (prior to the temperature change) to determine if this pre-incubation period depleted soil and litter of the more labile forms of C.

The priming effect of litter addition (and the interaction with temperature) was expressed as the percentage increase in cumulative soil-derived CO_2 mineralization in the litter treatments relative to the control at a particular temperature after day 15 of the incubation (after the temperature change):

$$\% \text{ PE} = \frac{100 \times (\text{Soil C}_{\text{Litter}} - \text{Soil C}_{\text{Control}})}{\text{Soil C}_{\text{Litter}}} \quad (6)$$

The temperature sensitivity of soil and litter-C decomposition was determined using Q_{10} values, calculated with the following equation:

$$Q_{10} = \left(\frac{C_{\text{HIGH}}}{C_{\text{LOW}}} \right)^{\left(\frac{10}{T_{\text{HIGH}} - T_{\text{LOW}}} \right)} \quad (7)$$

where C represents cumulative respiration (after the temperature change), T represents temperature (°C), and the subscripts high and low to incubation at higher and lower temperatures.

Carbon use efficiencies of litter and soil-derived C were estimated by the metabolic quotient ($q\text{CO}_2$), or the ratio of the respiration rate per unit biomass (Dilly and Munch, 1998). Microbial biomass was measured using PLFA yields converted to mg biomass-C with a conversion factor of 11.8 nmoles PLFA per mg biomass-C for fungi (Klamer and Bååth, 2004) and 363.6 nmol PLFA per mg biomass-C for bacteria (Frostegård and Bååth, 1996). The respiration rate during the 24 h preceding destructive harvesting of the particular sample measured for PLFA was used as the respiration rate. Microbial biomass and respiration rates used for calculating $q\text{CO}_2$ were partitioned into litter and soil-derived using the mixing model presented (Eq (3)). This measurement is a proxy for CUE, not true CUE due to (a) the conversion factor of PLFA to microbial biomass, (b) the unknown timeframe of CO_2 respiration that corresponds to the microbial biomass (here used as 24 h), and (c) the lack of measurement of the other potential transformations of C (e.g. exudates, enzymes). Therefore, while valid for comparison between litter and temperature treatments, $q\text{CO}_2$ cannot be directly compared to CUE from other studies.

2.4.3. Statistics

The influence of litter type and temperature upon cumulative C mineralization and soil priming effects was analyzed using two-way ANOVA with GenStat (16th edition) ($n = 4$ for each sampling day \times litter \times temperature treatment). For measurements of dissolved C and N and cellulase gene quantities at the destructive sampling measurements, we initially used a repeated measures ANOVA (with litter type and temperature as the between subject factors) to explore the data. To explicitly test for the effects of litter

type and temperature on particular sampling days, we used a two-way ANOVA on each sampling day. Changes in microbial community structure were analyzed using principal component analysis (PCA; proportional mol% of PLFA) or non-metric multidimensional spacing (MDS; T-RFLP data) in PRIMER (v6.1.15, Primer-E Ltd., Plymouth UK). An analysis of similarity (ANOSIM) in PRIMER was used to determine whether microbial community structure (PLFA, T-RFLP) grouped significantly based upon litter addition, sampling day, or temperature (Clarke, 1993). ANOSIM performs hypothesis testing through permutations of samples within user-specified groups (i.e. litter type or temperature). An R statistic is also provided to indicate the strength of the relationship; increasing R values from 0 to 1 indicate the strength of the dissimilarity between groups (groups are completely dissimilar with an R of 1). Changes in the isotopic composition of PLFA, total soil and litter-derived PLFA, and proportional soil and litter-derived PLFA were analyzed using repeated measured two-way ANOVA with litter type and temperature as the between subject factors. Tukey's post-hoc test was used for multiple comparisons. All data met conditions of normality except dissolved C and N, which was log transformed prior to analysis.

3. Results

3.1. Litter and soil chemistry

Using the molecular mixing model, each C source had a different C chemistry (Table 1; Suppl Fig. 1). In particular, the pre-incubated litter had proportionately more lipid-C than the fresh litter, while the fresh litter contained relatively more carbohydrate-C. The pre-incubated litter also had a lower % lignin: %N ratio (7) than the fresh litter (17), although both were lower than the soil (515). The C:N ratios of the two litters were lower than the soil, and the pre-incubated litter lower than the fresh litter. Therefore, the addition of C at a rate of 10 mg litter-C per g soil-C resulted in N additions of 17 mg fresh litter N per g of soil N and 38 mg pre-incubated litter N per g soil N, slightly decreasing the C:N ratio of the fresh litter + soil mix to 27.88 and the pre-incubated litter + soil mix to 27.18 relative to the starting soil (28.1).

3.2. Partitioning of soil and litter-derived C into measured C pools

3.2.1. Respired CO₂

At each incubation temperature, significantly more of the fresh litter was decomposed relative to the pre-incubated litter (Fig. 1a). Fresh litter decomposition increased significantly with each temperature step, while there was no significant impact of temperature on pre-incubated litter decomposition.

The extent of soil-C decomposition also varied with litter addition and temperature (Fig. 1b). Cumulative soil-C decomposition increased significantly with temperature in the fresh and pre-incubated litter treatments, while in the control the increase from 12 to 22 °C was not significant (significance not shown). Prior to the temperature change, there was significant positive soil-C priming with pre-incubated litter addition (+30 ± 16%) and significant negative soil-C priming with fresh litter addition (−58 ± 9.5%). After the temperature change on day 14, there was significant positive soil-C priming at 32 °C with pre-incubated litter addition and negative priming at 12 °C and 22 °C with fresh litter addition (Fig. 2). Increasing incubation temperature increased positive priming with pre-incubated litter addition and decreased negative priming with fresh litter addition, revealing that both temperature and litter addition impacted the extent of soil-C decomposition. The water content of the soils was not impacted by temperature, litter type, or sampling day (data are not shown).

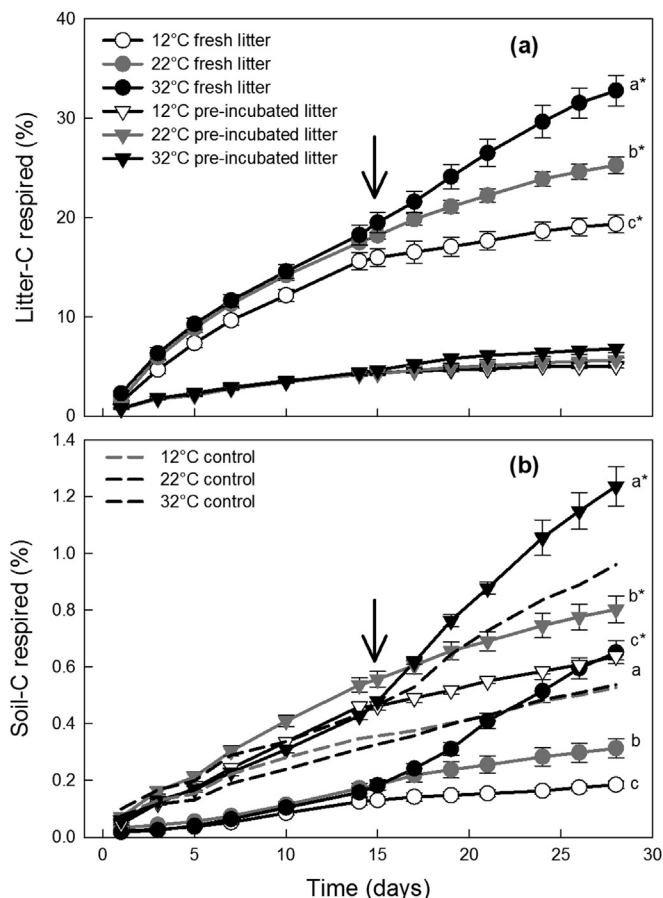


Fig. 1. Cumulative respiration of (a) litter-derived C and (b) soil-derived C. Letters indicate significant differences between temperature treatments while symbols indicate significant differences between litter treatments (significance for the control is not shown). Standard errors and sampling points are shown for the litter treatments but are omitted from the control (lines only) to improve the clarity of the figure. The arrow indicates the first sampling day after the temperature change.

The modeled MRTs of the more rapidly cycling pool were similar for the litters and soils at 7–8 days of incubation at 22 °C, confirming that the most labile portions of SOM and litter were degraded during the 14 days prior to the change in incubation

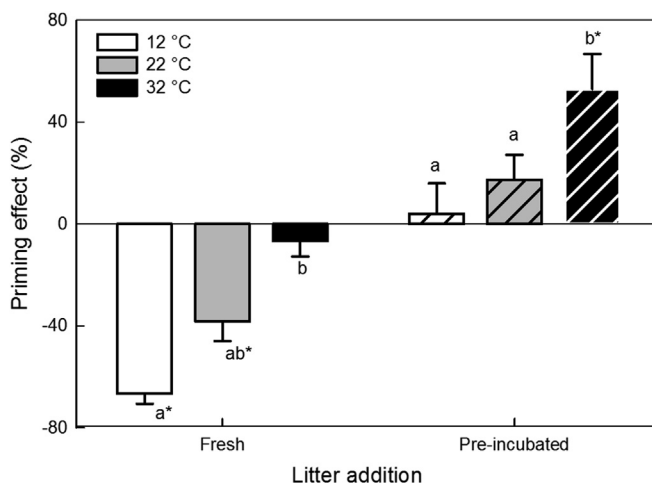


Fig. 2. Priming effect (% increase) of litter addition on soil-C respiration from days 15–28 of the incubation (after the temperature change). Values represent means ± standard errors. Asterisks indicate a significant priming effect; letters indicate significant differences between temperatures.

temperature (Suppl Table 1). Therefore, any changes in Q_{10} values of soil and litter-decomposition are not due to changes in the temperature response of the most actively cycling pool of C. The two litters had statistically similar Q_{10} values, suggesting they were equally temperature sensitive (Table 2). Soil-C was significantly more temperature sensitive than litter-C, and the litter additions significantly increased this soil-C temperature sensitivity.

3.2.2. Water-extractable DOM

There was significantly more fresh litter WEOC relative to pre-incubated litter WEOC (Fig. 3). While pre-incubated litter WEOC remained constant and insensitive to incubation temperature, fresh litter WEOC decreased significantly with temperature on days 19 and 28, but not on day 15 (24 h after the temperature change). In direct contrast to litter WEOC, there was 30% more soil DOC with pre-incubated litter addition relative to fresh litter and the soil only control (Table 3). There was no effect of temperature on soil-derived WEOC (data are not shown).

Differences in dissolved N between litter treatments were driven by ammonium, which was three to five times higher in the pre-incubated litter treatment (Table 3). Nitrate was also higher in the pre-incubated litter treatment relative to the control, although neither was different from the fresh litter treatment. Conversely, DON was higher in the fresh litter treatment relative to the pre-incubated litter treatment, although neither was different from the control. There were no significant impacts of temperature on dissolved N (data are not shown).

3.2.3. Microbial biomass yields

The control soil on average had lower total microbial biomass than the fresh and pre-incubated litter treatments (33 ± 4 vs. 62 ± 4 and 70 ± 4 nmol PLFA g soil⁻¹, respectively). These differences resulted entirely from similar production of litter-derived microbial biomass with fresh and pre-incubated litter addition (38 ± 2.0 and 33 ± 1.8 nmol PLFA g soil⁻¹, respectively), while soil-derived microbial biomass was significantly lower in the fresh litter treatment (24 ± 1.7 nmol PLFA g soil⁻¹) relative to the pre-incubated litter treatment and the control (37 ± 2.3 and 33 ± 3.9 nmol PLFA g soil⁻¹, respectively). Although total soil-derived microbial biomass yields were similar between the pre-incubated litter treatment and the control, the distribution of microbial groups varied, with significantly more gram-positive and gram-negative bacteria and significantly fewer fungi derived from soil-C in the pre-incubated litter treatment relative to the control (Fig. 4). The fresh litter treatment, with lower total soil-derived microbial biomass, had generally lower yields within the microbial groups, although there was a similar amount of gram-positive and gram-negative soil-derived biomass relative to the control. As litter-derived microbial biomass yields were similar between the two litter treatments, so were the yields of microbial groups, although there was significantly more litter-derived C in general microbial biomarkers derived from fresh

Table 2

Q_{10} values of litter and soil-C respiration. Values represent means \pm standard errors. Letters indicate significant differences between treatments.

Litter treatment	Q_{10}
Litter-C	
Fresh	1.89 \pm 0.19 a
Pre-incubated	1.68 \pm 0.18 a
Soil-C	
Fresh	3.49 \pm 0.49 b
Pre-incubated	3.03 \pm 0.37 b
Control	2.33 \pm 0.10 c

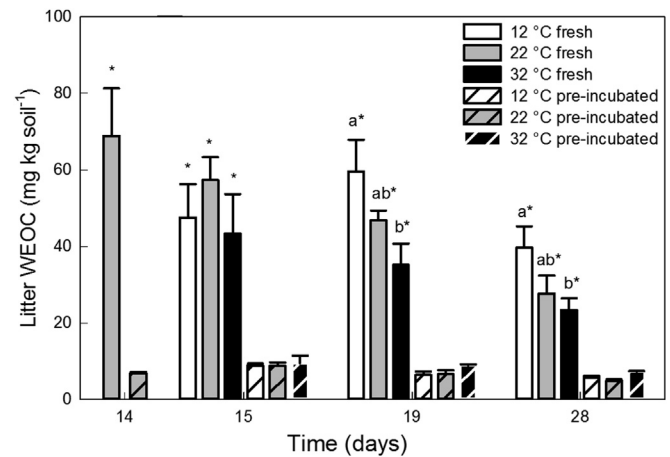


Fig. 3. Quantity of litter-derived WEOC. Values represent means \pm standard errors. Symbols indicate significant differences between litter types within a temperature, and letters between temperatures within a litter type. Day 14 was sampled prior to the temperature change and is included for reference.

Table 3

Concentration (mg kg soil⁻¹) of soil-derived water-extractable dissolved C and N in the litter treatments and control. Values represent means \pm standard errors. Letters indicate significant differences between treatments.

Litter treatment	WEOC	NH ₄ ⁺	NO ₃ ⁻	DON
Fresh	79 \pm 5 a	0.65 \pm 0.07 a	0.86 \pm 0.09 ab	2.16 \pm 0.33 a
Pre-incubated	107 \pm 5 b	3.52 \pm 0.19 b	1.09 \pm 0.11 a	1.78 \pm 0.33 b
Control	80 \pm 7 a	1.14 \pm 0.10 a	0.66 \pm 0.13 b	1.82 \pm 0.48 ab

litter relative to pre-incubated litter. There was no significant impact of temperature on biomass yields (data are not shown).

3.3. Microbial community structure

In addition to changes in total microbial biomass yields (Fig. 4), there was an impact of litter addition upon the structure of the microbial community, with proportionally more fungi in the litter treatments, more gram-positive bacteria in the control, and more general microorganisms in the fresh litter treatment (Fig. 5a, Suppl

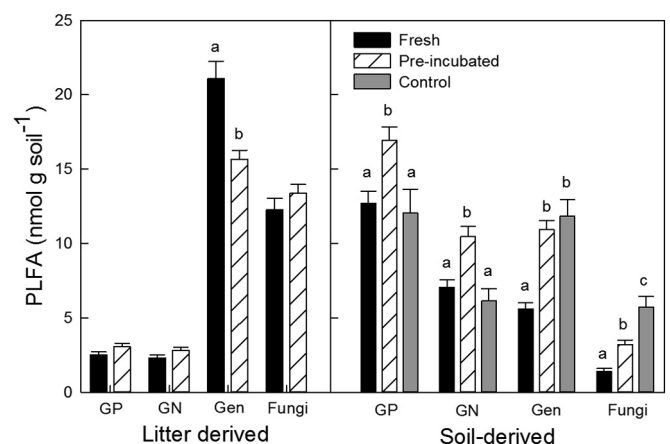


Fig. 4. Total litter-derived (left panel) and soil-derived (right panel) microbial biomass yields for broad microbial groups. Values represent means \pm standard errors. Letters indicate significantly different yields for a microbial group between litter treatments. GP = gram-positive bacteria, GN = gram-negative bacteria, Gen = general microorganisms.

Table 2 ($R = 1$ using ANOSIM). The main separation of the fresh and pre-incubated litter treatments along PC1 reveals that differences between the fresh and pre-incubated litter treatments were driving most of the variation in the data, while the more minor separation of the control and litter treatments along PC2 was driving less of the variation. However, this figure provides no information as to whether the differences between the treatments were due to microorganisms utilizing soil-derived or litter-derived C.

Therefore, to obtain a profile of the communities assimilating only litter-C, the proportional mol% contribution of an individual PLFA to the litter PLFA pool was determined by dividing the litter PLFA yield for an individual PLFA by the total litter PLFA within a sample. This process was repeated for soil-derived PLFA, and the calculated proportional mol % of litter-derived and soil-derived PLFA were analyzed together using PCA (Fig. 5b). From this, clear distinctions could be observed between the communities degrading soil- and litter-derived C, with proportionally more fungi and general microorganisms utilizing litter-derived C and more gram-positive bacteria utilizing soil-derived C ($R = 1$ using ANOSIM, between soil and litter communities; Suppl Table 2). There were also clear separations of the microbial communities

degrading fresh and pre-incubated litter ($R = 0.92$), and of the communities degrading soil-C in the control and litter treatments. As the microbial community in the soil prior to litter addition (original soil, Fig. 5b) was distinct from the communities degrading soil-C in the litter treatments but not in the control, the litter additions altered the structure of the microbial communities assimilating soil-C. The fresh litter addition resulted in the largest change in the soil microbial community relative to the control ($R = 1$), while the control and pre-incubated litter treatment soil utilizing microbes were slightly more similar but still distinct ($R = 0.92$). As groupings in the PCA were predominately driven by differences between the communities assimilating soil- or litter-derived C (rather than differences within litter and soil-C assimilation), proportional mol % distributions of the microbial groups utilizing soil-C or litter-C were examined separately.

As suggested by the PCA (Fig. 5b), litter-C decomposition was dominated by fungi and general microorganisms, with these two groups comprising 83–88 % of the total litter-derived microbial biomass (Fig. 6a, Suppl Table 2). However, the distribution of litter-C among the various microbial groups still varied significantly with litter type, with more general microorganisms assimilating fresh litter-C and significantly more fungi and gram-negative bacteria assimilating pre-incubated litter-C. Despite these changes, the ratio of gram-positive to gram-negative bacteria (GP:GN) and the ratio of fungi to gram-positive and gram-negative bacteria (fungi: bacteria; F:B) was consistent between litter treatments at around 1.1 and 2.4, respectively. Only gram-positive bacteria responded significantly to temperature, increasing in the fresh litter but decreasing in the pre-incubated litter with temperature, so there were relatively more litter-derived gram-positive bacteria assimilating pre-incubated litter relative to fresh litter at 12 °C and 22 °C but not at 32 °C (Suppl Table 2).

In contrast to litter decomposition, the proportion of soil-C held in microbial groups increased as follows: fungi < gram-negative bacteria < general microorganisms < gram-positive bacteria, with gram-positive bacteria comprising 35–50 % of total soil-derived microbial biomass (Fig. 6b). Litter type significantly impacted the distribution of soil-derived microbial biomass between microbial groups, with the proportional contributions of gram-positive and gram-negative bacteria increasing significantly as follows: control < fresh litter treatment < pre-incubated litter treatment, and with fungi and general microorganisms showing an opposite trend (Suppl Table 2). Differences in the abundances of microbial groups resulted in a significant decrease in the ratio of F:B assimilating soil-C from the control (0.32 ± 0.01) to the pre-incubated litter treatment (0.11 ± 0.01) to the fresh litter treatment (0.06 ± 0.01). Similarly, the soil-derived GP:GN ratio was highest in the control soil (2.0 ± 0.04) and decreased to the fresh litter treatment (1.8 ± 0.02) and then to the pre-incubated litter treatment (1.7 ± 0.04). Only gram-positive and general bacteria showed significant responses to soil-derived proportional yields with temperature, with an increase in gram-positive bacteria from 12 to 32 °C and a decrease in general bacteria from 12 to 32 °C (although neither were different from 22 °C).

Although PLFA are useful for linking microbial community structure with function, they have less resolution than DNA-based measurements and so the two can be complementary for analyzing microbial community structure. Bacterial T-RFLP profiles from the control soil and pre-incubated litter treatment grouped together and were statistically similar ($R = 0.02$; $p = 0.6$) while the fresh litter treatment was more variable and significantly different from both the pre-incubated litter treatment ($R = 0.50$, $p = 0.001$) and the control ($R = 0.39$, $p = 0.006$) (Fig. 7; 2D stress = 0.183).

The abundance of a cellulose-encoding gene (GH48) present in gram-positive bacteria (Actinobacteria) was significantly higher in

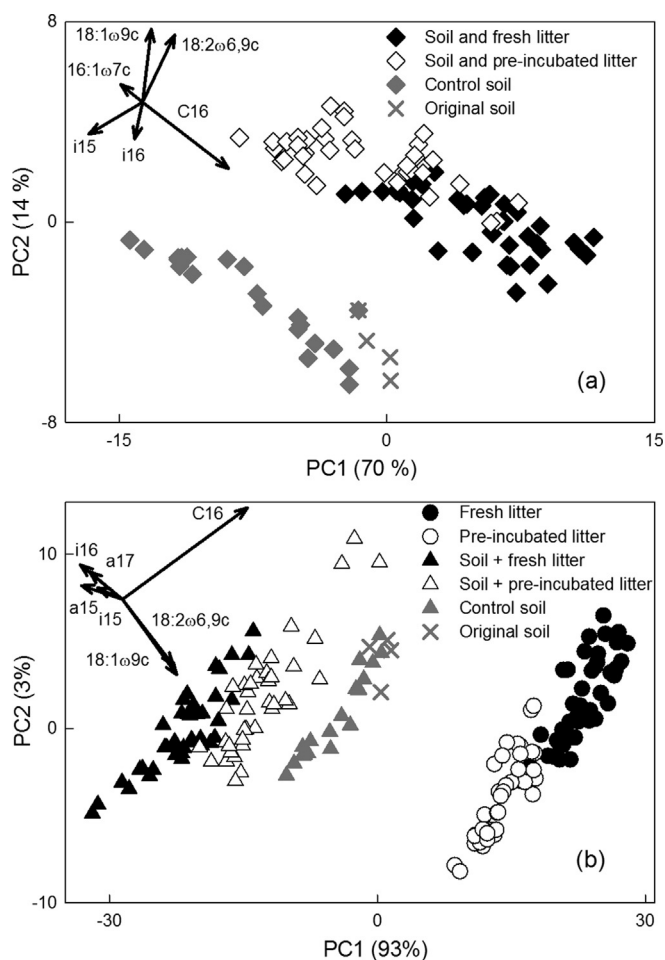


Fig. 5. Principal component analysis (PCA) of (a) the proportional mol % of the total microbial community and (b) the proportional mol % of microbial communities assimilating soil-C (circles) and the proportional mol % of the microbial communities assimilating litter-C (triangles), based upon the measured $\delta^{13}\text{C}$ atom fraction of individual PLFA. The percentage variance explained by each PC is shown in parentheses. The eigenvectors with the strongest correlations are shown in the upper left-hand corner of the figure; the length of the arrows is proportional to the strength of the correlation.

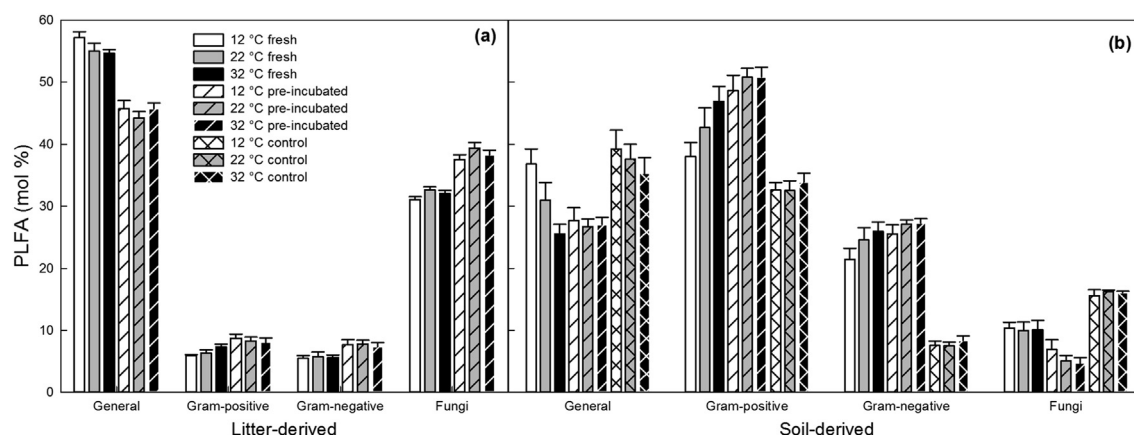


Fig. 6. Proportional mol % of total (a) litter-derived and (b) soil-derived PLFA held within microbial groups. Values represent means \pm standard errors. Significance is not shown to improve clarity of the figure and due to the presence predominately of main effects without interactions.

the pre-incubated litter treatment ($5.4e^{+5} \pm 5.1e^{+4}$ copies g soil $^{-1}$) relative to the fresh litter treatment and the control ($3.3e^{+5} \pm 3.8e^{+4}$ and $2.3e^{+5} \pm 3.4e^{+4}$ copies g soil $^{-1}$, respectively). However, when normalized to either the total amount of bacteria (16S) or total DNA present, there was no significant impact of litter type (data are not shown), suggesting that there were absolute but not relative increases in cellulose degradation by gram-positive bacteria in the pre-incubated litter treatment. There was no significant impact of temperature upon cellulase gene abundance.

3.4. Microbial metabolic quotient

In general, qCO_2 increased with temperature (greater CO_2 release per unit biomass), although the increase was not significant for pre-incubated litter at any temperature or for the soil from 12 °C to 22 °C with the litter additions (Table 4). Total qCO_2 was significantly lower in the pre-incubated treatment relative to the fresh litter treatment at 22 °C and 32 °C but not 12 °C. This was driven by the significantly lower qCO_2 of pre-incubated litter. Soil qCO_2 was higher in the control (highest CO_2 release per unit biomass) relative to the two litter treatments at 22 °C and 32 °C.

4. Discussion

The ability to partition measured C sources into litter and soil-derived pools provided some interesting insights into the

turnover of litter and soil-derived OM in response to both litter quality and temperature in this experiment (Fig. 8). In particular, there was a relationship between WEOC and C mineralization (but not a relationship between WEOC and microbial biomass) that was most strongly expressed in the litter-derived C pools. Pre-incubated litter WEOC and C mineralization was low and unresponsive to incubation temperature, while fresh litter WEOC and C mineralization was high and responsive to temperature. The >24 h lag between the temperature change and the response of fresh-litter WEOC (Fig. 3) suggests that biotic processes, most likely increased mineralization, drove the decrease in fresh-litter WEOC with temperature. The amount of soil-derived WEOC also partially reflected the extent of mineralization, as the pre-incubated litter treatment had the highest content of soil-derived WEOC and the greatest soil-C mineralization. However, a combination of increased desorption but also increased mineralization of soil-derived WEOC with temperature may have masked any response to temperature (Bengtson and Bengtsson, 2007; Conant et al., 2011). Similar to other studies, these data suggest that the mineralization of C (but not necessarily biomass formation) can be related to the amount of WEOC produced via enzymatic decomposition of insoluble OM (Marschner and Bredow, 2002; Kalbitz et al., 2003).

In contrast, soil and litter-derived microbial biomass total yields were generally unresponsive to temperature and relatively consistent across treatments, except for the lower yields of soil-derived microbial biomass from the fresh litter treatment (Fig. 8).

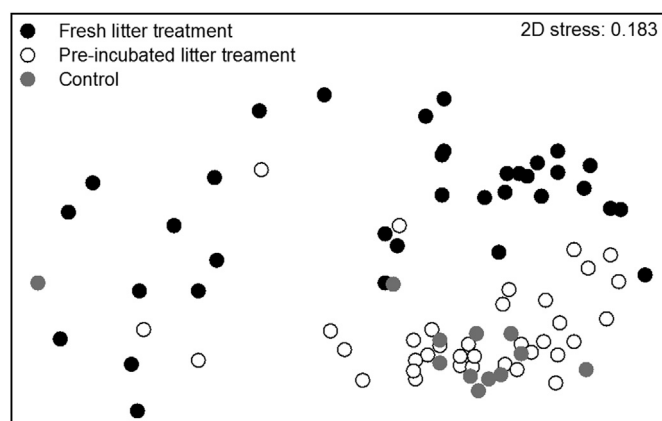


Fig. 7. Non-metric multiple dimension al scaling (MDS) of bacterial community T-RFLP profiles.

Table 4

Metabolic quotients (qCO_2) for soil-derived and litter-derived C. Values represent means \pm standard errors. Letters indicate significant differences between incubation temperatures within a litter treatment while symbols indicate differences between litter treatments within a temperature. Higher qCO_2 values indicate greater CO_2 release per unit biomass (lower carbon use efficiency).

Litter	Temp (°C)	Total qCO_2	Soil qCO_2	Litter qCO_2
Fresh	12	0.66 ± 0.07 a	0.32 ± 0.04 a	0.88 ± 0.11 a
	22	1.28 ± 0.11 b	0.58 ± 0.07 a	1.74 ± 0.18 b
	32	2.78 ± 0.22 c	1.81 ± 0.21 b	3.43 ± 0.30 c
Pre-incubated	12	0.43 ± 0.04 a	0.61 ± 0.06 a	0.23 ± 0.03 a*
	22	0.66 ± 0.09 a*	0.85 ± 0.14 a	0.46 ± 0.06 a*
	32	1.34 ± 0.14 b*	1.93 ± 0.15 b	0.73 ± 0.13 a*
Control	12	—	0.66 ± 0.13 a	—
	22	—	1.37 ± 0.21 b*	—
	32	—	2.72 ± 0.46 c*	—

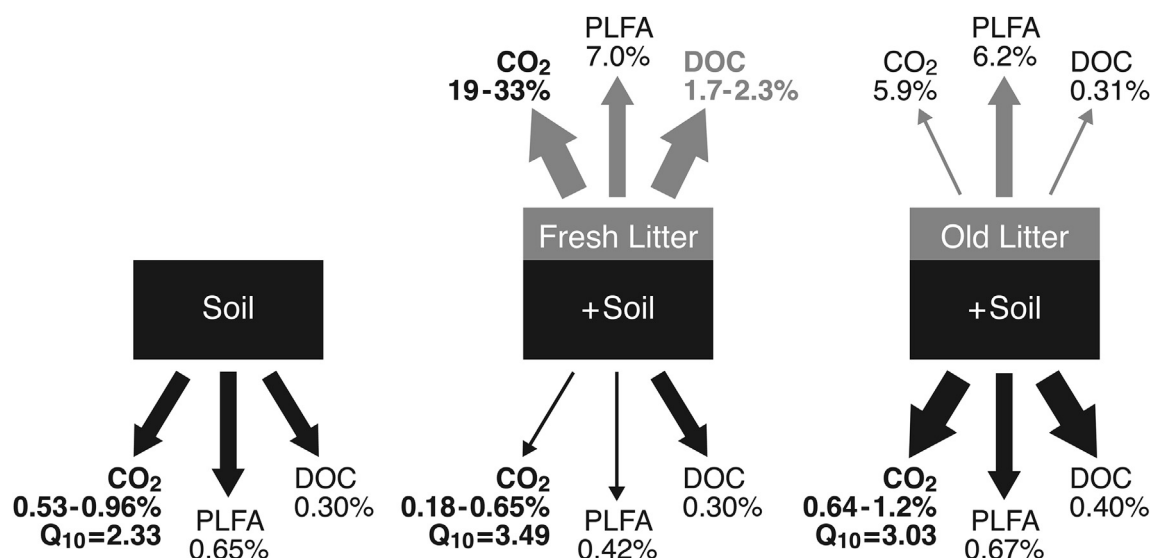


Fig. 8. Schematic of the flows of litter and soil-derived C pools from potential sources. The percentage of soil-C or litter C held in each pool is shown. Different size arrows for C fluxes denote a significant decrease (thin arrows) or increase (thick arrows) between litter treatments. An increase in pool size in response to temperature is shown in bold black text and a decrease in bold grey text. Temperature response of soil-C decomposition was stronger with litter addition relative to the control (higher Q_{10}). Changes in PLFA were in community composition not pool size.

However, the structure of the litter and soil-assimilating microbial communities did differ strongly (Fig. 5b), with carbon source utilization patterns of the various microbial groups (Fig. 6) reflecting previous knowledge regarding C substrate preferences, with fungi dominating litter decomposition and bacteria dominating soil decomposition (Strickland and Rousk, 2010; Gude et al., 2012). The higher soil-C assimilation of gram-positive bacteria relative to gram-negative bacteria is also consistent with previously published research confirming greater decomposition of soil-C by gram-positive bacteria (Waldrop and Firestone, 2004; Kramer and Gleixner, 2006, 2008; Bird et al., 2011).

In this experiment temperature and litter addition most strongly impacted CO₂ production and microbial community structure (Figs. 8 and 5b). This experiment has therefore provided clear evidence that litters with differing thermodynamic stability and C:N ratios can change both relative (Q_{10} ; Table 2) and absolute (priming; Fig. 2) measures of soil-C mineralization in response to temperature, by altering the structure and function of the microbial community. Differences in the magnitude of soil-C decomposition with litter addition could not arise from differences in SOC availability, chemistry, or edaphic properties, as the same soil was used in all incubations. Nor did it result from the recycling of soil-derived microbial biomass (apparent soil-C priming), as there were similar or lower yields of soil-derived microbial biomass with litter addition (Fig. 4). Instead, the addition of pre-incubated litter, which was more chemically similar to the soil (Table 1), promoted the growth of microorganisms (and presumably the production of extracellular enzymes) better equipped to degrade SOM, resulting in positive soil-C priming (Fig. 2). In contrast, the fresh litter, which had proportionately fewer lipids and more carbohydrates relative to the pre-incubated litter and the soil, promoted the growth of microorganisms less equipped to degrade SOM, resulting in negative priming. This was confirmed by two independent methods examining microbial community structure, as both the PLFA profiles of the microbial communities utilizing soil-C (Fig. 5b) and the T-RFLP profiles of the bacterial communities (Fig. 7) were more similar between the pre-incubated litter treatment and the control than the fresh litter treatment. The greater dominance of both gram-positive and gram-negative

bacteria decomposing soil-C in the litter addition treatments relative to the control (Fig. 6), and the higher gram-positive and gram-negative soil-derived biomass yields in the pre-incubated treatment (which had positive priming), suggests that bacteria may have been driving some of the observed soil priming response with litter addition.

Despite the differences in the direction of priming observed with litter addition, we observed a consistent increase in the priming response to temperature (Fig. 2). Soil priming has been attributed to the activity of certain microbes (particularly fungi) more than others (De Graaff et al., 2010; Fontaine et al., 2011; Garcia-Pausas and Paterson, 2011; Paterson and Sim, 2013), although gram-positive bacteria have also been implicated (Bird et al., 2011). We did not find evidence for fungi mediating changes in priming with litter addition or temperature in this study, but we did observe significant changes in microbial community structure with temperature, particularly a consistent increase in soil-utilizing gram-positive bacteria (Fig. 6, Suppl Table 2). Although other groups (particularly gram-negative bacteria) may also have contributed to differences in the direction of priming between litter treatments, only gram-positive bacteria showed a consistent response to temperature. A meta-analysis by Allison and Martiny (2008) found that the majority (80%) of soil-warming experiments showed changes in microbial community structure, and many studies (although not all) show increases in gram-positive bacteria (Zogg et al., 1997; Rinnan et al., 2007; Frey et al., 2008; Feng and Simpson, 2009; Schindlbacher et al., 2011; Ziegler et al., 2013; Wei et al., 2014; Xiong et al., 2014); a microbial group associated with soil-C decomposition in this study and often associated with mineral C decomposition and low substrate availability (Fierer et al., 2003; Kramer and Gleixner, 2008). The results from our study suggest that this microbial group may potentially mediate the response of soil-C mineralization and priming in a warming climate. In particular, if gram-positive bacteria have different carbon use efficiencies than other groups, or if their biomass is stabilized more or less strongly in soil (Thiet et al., 2006; Throckmorton et al., 2012; Malik et al., 2013), changes in their abundance could further impact downstream soil-C cycling in response to climate change.

There was also a potential for an influence of N upon SOM and litter decomposition, due to the lower C:N ratios of the litters relative to the soil (Table 1). However, the addition of N did not cause the priming response in this experiment, as (a) the two litters caused different directions of priming, and (b) the substrate with the lowest C:N ratio (Table 1) and highest inorganic N concentrations (Table 3) had the strongest soil-priming response (Fig. 2), inconsistent with a mechanism of priming driven by microbial 'mining' of the soil for N after the addition of C (Fontaine et al., 2003; Moorhead and Sinsabaugh, 2006; Craine et al., 2007). However, the litter additions may have impacted the increase in priming in response to soil temperature, as the higher N content of the soil-litter mixtures may have allowed for the production of a larger number of hydrolytic enzymes (Waldrop et al., 2004; Cusack et al., 2011), or a suite of more efficient enzymes (Stone et al., 2012), that were capable of degrading more soil-C as the temperature increased. Whether N availability can interact with temperature to influence the extent of soil-C priming warrants further investigation.

In our study, differences in microbial CUE (represented by qCO_2) were not controlled by thermodynamic stability (e.g. Frey et al., 2013), as microbes utilizing the thermodynamically stable pre-incubated litter were the most carbon-use efficient and less responsive to temperature, while microbes utilizing the thermodynamically labile fresh litter were the least carbon-use efficient (Tables 1 and 4). Instead, microbial community structure and N availability may have controlled this response. Faster-growing, zymogenous microbes (r-strategists) have lower CUE than slower-growing autochthonous microbes (K-strategists), while N limitation can decrease CUE as excess C is respired as CO_2 rather than used to build more microbial biomass (Manzoni et al., 2008, 2012). If driven solely by C:N ratios, then the CUE should have increased from the soil < fresh litter < pre-incubated litter. The pre-incubated litter, with a C:N ratio nearing the ratios required for microbial biomass (Cleveland and Liptzin, 2007; Manzoni et al., 2010), did have the highest CUE (lowest qCO_2) (Table 4). Similarly, the higher CUE of the soils with litter addition relative to the control soil may have been driven by more available N resulting from the litter addition (Table 3). Differences in microbial community structure were likely not causing the higher CUE of the soils with litter addition relative to the control, as the control soil had the highest ratios of gram-positive bacteria to gram-negative bacteria (GP:GN) and fungi to total bacteria (F:B), a rough proxy for the ratio of K to r-strategists (De Vries and Shade, 2013). However, the fresh litter had a low CUE (high qCO_2) that may have been controlled by microbial community structure, as the high proportion of carbohydrate-C (Table 1) may have allowed for the enhanced growth of r-strategists, although there was no evidence for this in F:B or GP:GN ratios. In the pre-incubated litter, the lack increase in CUE with temperature suggests that a mechanism in addition to greater N could be controlling this response. Although microbial CUE of higher quality substrates is unresponsive to temperature (Frey et al., 2013), the pre-incubated litter contained more low-quality substrates (Table 1) and therefore should have lower CUE. Alternatively, recycling of microbial biomass results in high microbial CUE (Schimel and Weintraub, 2003; Manzoni et al., 2012), and increased microbial turnover has been observed with warming (Hagerty et al., 2014). The greater proportion of lipid-derived C in the pre-incubated litter (Table 1) was either derived from the selective enrichment of plant-derived lipids (e.g. Feng et al., 2008) or increases from dead microbial biomass formed during the pre-incubation (Baldock et al., 1990); if derived from microbial biomass this could have contributed to the low qCO_2 (high CUE) in the pre-incubated litter and the lack of increase with temperature. If so, this supports the concept that C funneled preferentially into

microbial biomass, rather than respired as CO_2 , may allow for C stabilization as temperatures increase, if it continues to be efficiently recycled by subsequent microbial populations.

There have been a number of recent studies highlighting the importance of microbial community composition on the resulting extent and chemistry of decomposing litter and soil C (Balser and Firestone, 2005; Wickings et al., 2012; Allison et al., 2013). Our study adds to these results by showing that the extent of soil-C mineralization, the response of soil-C decomposition to temperature, and the allocation of C between microbial biomass and CO_2 , can be altered in one soil as a result of different microbial communities degrading soil C. As microbial community structure was influenced by the chemistry of the litter inputs, the predicted changes in above and belowground plant inputs and chemistry (Pendall et al., 2004) due to higher atmospheric CO_2 concentrations have the potential to alter microbial community structure, which (as this study has shown) can impact both the absolute and relative response of CO_2 decomposition to temperature. It is therefore important to continue to characterize the response of belowground communities to global change scenarios (elevated CO_2 , N deposition, warming, and land use change) in varying environments where the microbial community may be adapted to different conditions. For example, the dominance of gram-positive bacteria over the temperature response of the priming effect in this experiment may in part result from the soils sampled from a relatively warm, drought-prone, and N-limited system that could favor the growth of gram-positive bacteria (Schimel et al., 2007), where in N-rich or cool, wet systems different microbial groups (such as fungi) may dominate priming and temperature responses (e.g. Ziegler et al., 2013). It is therefore necessary to determine whether the importance of microbial community structure in altering the response of SOM decomposition to temperature is consistent in other environments that may have higher prevalence of functionally distinct microbial groups. Studies that address changes in the microbial community, and resulting changes in function, across broad environmental gradients are therefore warranted, and could potentially aid more recent efforts in incorporating microbial attributes into litter and soil decomposition models (Allison, 2012; Moorhead et al., 2012; Wieder et al., 2013). For example, examining the fate of ^{14}C or ^{13}C labeled substrates across varying climates and N availability may help determine whether microbial community structure is consistently important in dictating the response of SOC decomposition to temperature, as observed in this experiment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2014.10.008>.

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